American Journal of Hematology 57:212–214 (1998)

Presence of Allele α^{LELY} in an Amazonian Indian Population

Daniela S. Bassères,[1,3] Tereza S.I. Salles,[1] Fernando F. Costa,[1,2] and Sara T.O. Saad[1,2]*

[1]Hemocentro, Universidade Estadual de Campinas, Campinas, SP, Brazil
[2]Department of Medical Clinic, Universidade Estadual de Campinas, Campinas, SP, Brazil
[3]Department of Genetics and Evolution, Universidade Estadual de Campinas, Campinas, SP, Brazil

Allele α^{LELY} is a low-expression allele of the erythroid spectrin α-chain that is characterized by a C → G mutation at position α1857 in exon 40 and a C → T (nt −12) mutation in intron 45. This second mutation is probably responsible for the partial skipping of exon 46. This exon is essential for the nucleation of the α-chains by the β-chains during erythropoeisis. Although allele α^{LELY} remains asymptomatic in both heterozygotes and homozygotes, it enhances the expression of deleterious α-alleles that occur and, as such, has clinical importance. In this study, the frequency of allele α^{LELY} was estimated in two ethnically different Brazilian populations: a random sample of blood donors from Campinas, a city located in São Paulo State, in the southeastern region of Brazil, and a sample of Parakanã Indians (Tupi tribe), a very isolated population with a high degree of inbreeding.

The frequency of allele α^{LELY} in the blood donor's sample (n = 54) was 24.1% whereas in the indigenous sample (n = 41), it was 15.9%. These frequencies were not significantly different at the 5% level ($\chi^2 = 1.931$). Similarly, when the frequencies of our samples were compared with those of the four ethnic groups studied by Maréchal et al. [Br J Haematol 90:553–556, 1995], no significant differences were found at the 5% level ($\chi^2 = 6.686$).

These results suggest that allele α^{LELY} is a very ancient allele since it occurs with a relatively uniform and high frequency in all human ethnic groups studied so far. These findings confirm the importance of allele α^{LELY} in influencing the expression of deleterious α-spectrin alleles. To our knowledge, these are the first data concerning allele α^{LELY} in native Americans. Am. J. Hematol. 57:212–214, 1998.

Key words: α-spectrin; allele α^{LELY}; South America; Amazonian Indians; Brazilian population

INTRODUCTION

Allele α^{LELY} is a low-expression allele of the erythroid spectrin α-chain. This allele was first characterized by an increased susceptibility to proteolysis of the αIV–αV domain junction, which resulted in an enhancement of the αV/41 kD fragment after the tryptic digestion of spectrin [1]. This increased susceptibility to proteolysis is due to a C → G mutation at position α1857 in exon 40 of the α-spectrin gene [2]. However, this mutation does not explain the low expression of allele α^{LELY}. A second mutation, C → T (nt −12), in intron 45 of the α-spectrin gene was subsequently found to be linked to the exon 40 defect with no single exception. This second mutation is probably responsible for the partial skipping of exon 46 during splicing and, therefore, for the low level of expression [2]. The skipping of exon 46 causes the deletion of six aminoacids in the nucleation site of the α-spectrin chain. Alfa-chains lacking these amino acids are not recruited by the β-chains during membrane assembly, and consequently, are not expressed [3]. Allele α^{LELY} remains asymptomatic in both heterozygotes and homozygotes because the α-chains are synthesized in a large excess relative to the β-chains, and the skipping of exon 46 is only partial. In contrast, allele α^{LELY} enhances the expression of deleterious α-alleles that occur and, as such, has clinical importance. The clinical significance of this allele, compared to other low-expression alleles,

Contract grant sponsor: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Contract grant sponsor: Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp).

*Correspondence to: Sara T.O. Saad, Hemocentro-UNICAMP, CP6198, Campinas, SP, Brazil. E-mail: sara@obelix.unicamp.br

Received for publication 10 April 1997; Accepted 8 October 1997

arises from its relatively high frequency in all populations analyzed [4].

The ethnic origin of the Brazilian population is heterogeneous and unevenly distributed within a country of continental dimensions. Besides the native population, Brazil has received immigrants from Italy, Spain, Germany, Japan, and the Middle East. During the slave trade, Brazil received about 4 million Africans, mainly from Angola, Congo, and Mozambique, who settled in almost all regions of the country [5].

The intense process of miscegenation throughout the country makes the Brazilian population unique in its ethnic background.

The Amerindians are unlike any other population of similar size or antiquity, in that they have undergone evolution in virtual isolation for 15,000–20,000 years [6]. The Parakanã Indians are derived from the Tupi tribe and first established contact with neo-Brazilians in 1971. These Indians constitute a very small population (about 347 individuals in 1984) and are scattered throughout three villages in the north of Pará State (northern Brazil). In relation to the mating strategies, this group is polygamous and favors marriage of males to their sister's daughters. As a result, the population is very inbred [7]. Therefore, the bottleneck effect, to which this population has been submitted during its evolution, is not counteracted by the broadening of their gene pool through exogamous gene flow.

Analysis of 13 biallelic systems in the Parakanã Indians shows that they are more distant from the South American Indian founders and from Europeans than 40 other tribes [7]. For this reason, it would not be surprising if the frequency of allele α^{LELY} was altered or if this allele was even absent or fixed in this population.

The aim of this study was to estimate the frequency of allele α^{LELY} in Parakanã Indians and to compare it with a general Brazilian population of mainly European and African ancestry.

METHODS

Populations Studied

The representative Brazilian population sample consisted of 54 unrelated blood donors attended at the Hematology and Hemotherapy Center of the State University of Campinas. The indigenous sample consisted of 41 individuals among whom it was only possible to exclude first-degree relationships. Informed consent was obtained from all subjects and the study was approved by the University Hospital Ethics Committee.

Detection of the Exon 40 Mutation

Genomic DNA was extracted from peripheral blood leukocytes by standard techniques using phenol-chloroform or NaCl. Exon 40 of the α-spectrin gene was amplified by the PCR [8] using primers 39 5′-CGTGAGTCTGAATATGAGCG-3′ and 40 5′-ATTCAGCCTCTATCTTGG-3′ [9]. The product of amplification was subsequently digested with the restriction enzyme Mwol since the α1857 C → G mutation abolishes a Mwol restriction site. The digested products were run in agarose gels stained with ethidium bromide and the digestion patterns were used to determine the presence or absence of the exon 40 mutation.

TABLE I. α^{LELY} Allele Frequency in Different Populations*

Population	Number of subjects examined	Frequency of allele α^{LELY} (%)
Present study		
Brazilian blood donors	54	24.1
Parakanã Indians	41	15.9
Marechal et al. [4] study		
Caucasians	52	32.7
African blacks	43	20.9
Japanese	50	20.0
Chinese	18	22.0

*Modified from Marechal et al., 1995 [4]. The differences between populations are not significant at the 5% level ($P > 0.05$).

Statistical Analysis

The Chi-square test was used to determine the significance of any difference between the α^{LELY} allele frequencies at the 5% level.

RESULTS

The frequency of the α^{LELY} allele in 54 unrelated blood donors (corresponding to 108 alleles) was 24.1%. Among the 82 alleles from 41 Indians, α^{LELY} polymorphism was present in 15.9%. The difference of nearly 10% between these two frequencies was not significant at the 5% level ($\chi^2 = 1.931$) (Table I). Comparison of the α^{LELY} allele frequencies of our samples with those of the four ethnic groups studied by Maréchal et al. [14] showed that there were also no significant differences at the 5% level ($\chi^2 = 6.686$) (Table I).

DISCUSSION

The Brazilian population is unique because of the intense miscigenation that occurs throughout the country. São Paulo is the most populated state and has been receiving migrants from all regions of the country, particularly from the Northeast. Campinas, a city of 1,000,000 inhabitants, is situated in the state of São Paulo and is the largest city in a region with a population of 5,000,000. This population is characterized by a high degree of racial mixture between European and African descendants [10]. For this reason, the blood donors from Campinas represent a good example of a population submitted to

intense gene flow followed by admixture. The frequency of allele α^{LELY} in this population was 24.1% and was not significantly different from other populations already studied [4]. This finding indicates that the α^{LELY} allele has been maintaining uniform frequencies among several populations. Maréchal et al. [4] proposed that allele α^{LELY} is a stable allele, which suggests the action of stabilizing evolutionary forces. However, as this allele is supposedly neutral, stabilizing natural selection should have no influence in maintaining allele α^{LELY} frequency uniform. It is also hard to imagine that this allele, if submitted to selective forces, would have the same selective values around the world, thus leading to the same allele frequency. This stability, nevertheless, can be explained by the action of gene flow, which is markedly intense in the population of Campinas.

Among the Parakanã Amerindians, the frequency of the α^{LELY} allele was 15.9%, which was not significantly different from those estimated for other ethnic groups. The presence of allele α^{LELY} in an Amerindian group assures us that this allele is indeed very ancient, as proposed by Maréchal et al. [4], and emerged before the migration of Polynesians to America. To our knowledge, these are the first data concerning allele α^{LELY} in a native American population.

The Parakanã Indians have a very restricted range of polymorphisms for several genetic markers, including blood groups, HLA system, hemoglobin, red cell enzymes, serum protein phenotypes, etc. [7]. The alleles I^0 (ABO system), k (Kell system), Hb^A (hemoglobin system), Gd^B (G-6-PD system), PGD^A (6PGD system), EsA^1 (Esterases A_1–A_3,B system), $PGM^1{}_2$ (phosphoglucomutase2 system), AK^1 (adenylate kinase system), ADA^1 (adenosine deaminase system), $PGM^1{}_1$ (phosphoglucomutase1 system), Cp^B (ceruloplasmin^2 system), Fy^a (Duffy system), all have frequencies of 100% or nearly so. The allele L^N (MNSs system) is absent in the population. These values show the great loss of variability that occurred in this very isolated population and demonstrate how much they differ from other populations.

These distinctive features may have arisen through a combination of founder effects and genetic drift. Only a limited variety of genes was brought to the continent by migrants over the Bering Bridge and isthmuses of Central America. In addition, tribal formation occurs by the growth of groups initially segregated as villages. New villages form by the fission of old ones along familial lines and, thus, segregate out relatively small numbers of alleles [11,12].

Our results indicate that the frequency of allele α^{LELY} has not been altered significantly by the successive founder effects that led to the formation of the Parakanã Indian population. If we assume that, at the moment of the foundation, the frequency of this allele remained unchanged, we can come to the conclusion that, afterwards, the genetic drift has not altered allele α^{LELY} frequency significantly, probably because there has not been enough time for its action to be effective at this locus. However, we cannot rule out the possibility that our sample was not large enough and, therefore, that an existing significant difference may not have been detected.

The findings of this study support the opinion that allele α^{LELY} is homogeneously distributed throughout the world. They also support the hypothesis that gene flow may act as an important factor in maintaining allele α^{LELY} frequency stable.

ACKNOWLEDGMENTS

We thank Dr. Louis-Bernard Klakzo for his relevant contribution to this work, through his stimulating questions and intriguing suggestions. This work was supported by the Conselho Nacional de Desenvolvimento Cientifico e Technológico (CNPq) and by the Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp).

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